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File: USPT

Oct 1, 1996

DOCUMENT-IDENTIFIER: US 5560923 A

TITLE: Method of encapsulating anthracycline in liposomes

Abstract Text (1):

A method of preparing a liposomal anthracycline glycoside composition, which entails forming cardiolipin-containing liposomes by drying a lipid mixture containing cardiolipin, and then introducing an aqueous solution, and mixing the cardiolipin-containing liposomes with a solution of anthracycline glycoside.

Brief Summary Text (8):

More recently, liposome delivery systems have been used to reduce anthracycline uptake in cardiac tissue, while preserving drug activity. See for example, U.S. Pat. No. 4,419,348. In using this methodology, it is found that doxorubicin in cardiolipin containing liposome exerts antitumor activity at doses that cause fewer myocardial alterations than the same dose of free doxorubicin. For example, prevention of doxorubicin cardiotoxicity in beagles by liposomal encapsulation has also been shown. Generally, the preparation of this form of doxorubicin involves a two-step preparation process, which entails complexing of the drug with cardiolipin and subsequently encapsulating the complex in liposomes. However, this preparation is very difficult to manage at an industrial level, particularly in conserving good encapsulation efficiency and preserving anthracycline glycoside stability.

Brief Summary Text (14):

Accordingly, the above objects and others which will become apparent in view of the following disclosure are provided by a method of preparing a liposomal anthracycline glycoside composition, which entails forming cardiolipin-containing liposomes by drying a lipid mixture containing cardiolipin, and then introducing an aqueous solution, and b) mixing the cardiolipin-containing liposomes with a solution of anthracycline glycoside.

Drawing Description Text (3):

FIG. 2 illustrates a comparison of cytotoxicity of free doxorubicin, liposome-encapsulated doxorubicin and cardiolipin-liposome-complex doxorubicin using LZ cells.

Drawing Description Text (4):

FIG. 3 illustrates survival curves of MCF-7/ADR cells treated with free doxorubicin, empty liposomes, cardiolipin-liposomes and doxorubicin in simultaneous combinations with empty cardiolipin-liposomes.

Detailed Description Text (4):

The present invention is, in part, directed to a liposomal anthracycline glycoside preparation which is based on a composition of one or more anthracycline glycosides and cardiolipin-containing liposomes. The present invention also pertains to a method of preparing such a composition.

Detailed Description Text (7):

In accordance with the present invention, the cardiolipin-containing liposomes may be formed by drying a lipid mixture containing cardiolipin and then introducing an aqueous solution. Dispersion is completed by strongly homogenizing the mixture

using a vortex, magnetic stirrer and/or sonication. The liposomes are negative liposomes considering the inherent negative charges of cardiolipin and that the lipid added to cardiolipin must be neutral or negative such as phosphatidyl choline, cholesterol, phosphatidyl serine or phosphatidyl glycerol. For better stability, however, the liposome preparation may be lyophilized.

Detailed Description Text (9):

The present invention is, in part, predicated upon the discovery that anthracycline glycosides may be complexed to the cardiolipin-containing liposomes prior to clinical administration. Surprisingly, complexation is achieved by simply mixing cardiolipin-containing liposomes concentrate with a solution of anthracycline glycoside or by rehydration. For example, doxorubicin may be diluted in a 0.90% NaCl solution with 0.0017M phosphate buffer at pH of 7.4.

Detailed Description Text (10):

The anthracycline composition may be prepared also by two additional methods: 1) by rehydrating lyophilized cardiolipin-containing liposomes with a solution of anthracycline glycoside, or 2) by rehydrating with the buffer the mixture of lyophilized liposomes and crystalline doxorubicin.

Detailed Description Text (11):

The two additional methods described above may be practiced as follows. First, lyophilized cardiolipin-containing liposomes may be rehydrated with a solution of anthracycline glycoside. The cardiolipin-containing liposomes may be lyophilized using conventional methodologies. The rehydrating solution of anthracycline glycoside will have a concentration of at least about 15 .mu.g/ml. Of course, all solutions described in the present specification are in pharmaceutically-acceptable solvents, such as saline solution of physiologically-acceptable concentration and pH and dextrose 5% saline.

Detailed Description Text (13):

Complexation of the anthracycline glycoside drug to cardiolipin-containing liposomes is due to the high binding affinity of such drugs, such as doxorubicin, to cardiolipin. From a pharmacological standpoint, the molar ratio of anthracycline glycoside to lipids may be adapted according to the type of tumor cells to be treated or the therapeutic end-point required. However, in order to obtain a good complexing efficiency, the cardiolipin content in liposomes should be at least half that of anthracycline glycoside added in terms of molar ratio. The complexed anthracycline glycoside-cardiolipin is strongly stabilized by an electrostatic interaction between two molecules of the glycoside and one molecule of cardiolipin and a stoichiometric interaction leading to a card pack dimer formation.

Detailed Description Text (18):

Preparation of a cardiolipin-containing liposome composition of the invention

Detailed Description Text (22):

Preparation of an Anthracycline Cardiolipin Containing liposome composition with Doxorubicin

Detailed Description Text (23):

Complex formation and integration of Doxorubicin into the lipid bilayer membrane of the cardiolipin-containing liposome was achieved prior to the clinical administration by simple vortex mixing of a vial containing 40 mg cardiolipin-liposome lyophilizate and 2.5 ml of a Doxorubicin solution previously prepared in 0.85% NaCl at 2 mg/ml. Vortex mixing is completed for 1 minute and mixture is kept at 37.degree. C. for a 15 min. period incubation. Doxorubicin HCl was obtained from Adria Laboratories.

Detailed Description Text (25):

Determination of the Preferred Conditions of Incubation for Complexing Cardiolipin-

Containing Liposomes to DoxorubicinDetailed Description Text (28):

Association capacity of doxorubicin to cardiolipin-liposomes was evaluated for different concentrations of mixture of both components mixed in the same volume (5 ml) after an incubation of 15 min. at 37.degree. C. at a fixed liposome/doxorubicin weight ratio of 11 (Table 1). The result obtained show that nearly all the drugs present in the incubation mixture was complexed to the cardiolipin-containing liposomes revealing an increase of complex formation when the incubation is carried out at 37.degree. C. compared to an incubation temperature of 25.degree. C. In addition, results demonstrate that even at a high dilution of both components (20 .mu.g/ml of Dox and 0.22 mg/ml of liposomes) complex formation is very effective and nearly complete.

Detailed Description Text (30):

Study of the in vitro Drug Delivery Efficiency by Cardiolipin-Liposomes-Complexed Doxorubicin. Effect on Multidrug Resistance.

Detailed Description Text (31):

Resistance to major classes of cytotoxic drugs may emerge in tumor cells from patients treated by chemotherapy. Therefore, multidrug resistance may be one therapeutic obstacle in cancer treatment. It has been shown that liposome-encapsulated doxorubicin may modulate multidrug resistance in cancer cells. (A. R. Thierry, T. J. Jorgensen, D. Forst, I. A. Belli, A. Dritschilo, A. Rahman. Modulation of Multidrug Resistance in Chinese Hamster Cells by Liposome-encapsulated Doxorubicin. Cancer Comm. Vol. 1 pp. 311-316. (1989)). The capability to increase Doxorubicin activity in multidrug resistant cells was due to the use of a liposomal carrier. This capability was studied when cardiolipin-liposome-complexed doxorubicin. Thus, multidrug resistance reversal ability bore witness to the integrity or stability of the cardiolipin-liposome-doxorubicin complex.

Detailed Description Text (32):

Clonogenic assay was performed to evaluate modulation of multidrug resistance of free doxorubicin, cardiolipin-liposome-complexed doxorubicin and liposome-encapsulated doxorubicin in MCF-7/ADR and LZ cells which are resistant to doxorubicin. MCF-7/ADR and LZ cells are multidrug resistant cell lines originating from human breast cancer and Chinese hamster fibroblast, respectively.

Detailed Description Text (34):

In FIG. 2, cytotoxicity of free doxorubicin, liposome-encapsulated doxorubicin, and the cardiolipin-liposome-complexed doxorubicin were compared using LZ cells. In Dox+Lip I treatment, cells were exposed to cardiolipin-liposome-complexed doxorubicin which was prepared from mixing drug to a concentrate of previously formed cardiolipin-liposomes. Dox+Lip II correspond to cardiolipin-liposome-complexed doxorubicin which was prepared from mixing drug to a cardiolipin-liposome lyophilizate. The three liposomal doxorubicin preparations used in this experiment exhibited comparable cytotoxicity against LZ cells and thus a comparable drug resistance reversal capacity (approximately 9-fold compared to free drug in terms of IC.sub.50).

Detailed Description Text (35):

Survival curves of MCF-7/ADR cells treated with free Doxorubicin, empty liposomes, cardiolipin-liposomes and doxorubicin in simultaneous combinations with empty cardiolipin-liposomes are shown in FIG. 3. In this experiment cardiolipin-liposomes were complexed with free doxorubicin directly in the culture medium (volume 5 ml) before cell treatment and cardiolipin-liposomes concentration added to the drug was equal to that present at equivalent doxorubicin concentration in liposome-encapsulated doxorubicin. Both liposomal doxorubicin preparations present a comparable cytotoxic effect and thus a comparable drug resistance reversal effect. It was established in the human breast carcinoma resistant MCF-7/ADR cell line that

both liposomal doxorubicin preparations have the same cytotoxic activity revealing that the drug in the cardiolipin-liposome-complex is as well integrated as in the liposome-encapsulated drug.

Detailed Description Text (36):

FIG. 4 shows the reversal capacity of various treatments in the LZ cells. Generally, reversal capacity to multidrug resistance refers to the level at which drug resistance is overcome and is measured by the ratio of these amounts for free drug/liposome-encapsulated drug. For example, if 5 mg of free drug or 1 mg of liposome-encapsulated drug are required to overcome resistance to a drug, a reversal capacity of 5 is indicated. This would generally indicate that the liposome-encapsulated drug is five times as effective as the free drug in a drug-resistant host. Results demonstrate that increased concentrations of liposome in combination with Doxorubicin, for example substantially enhance the cytotoxic effect of the drug. As shown previously, liposome encapsulated doxorubicin seems to be approximately as cytotoxic as cardiolipin-liposome-complexed doxorubicin when liposome concentration (0.2 mg/ml) added to drug was equal to that present at equivalent drug concentration. For example, when 0.6 mg/ml and 1.0 mg/ml of liposomes (concentration corresponding to 90% and 50% survival for liposome cytotoxicity alone in LZ cells) are added to doxorubicin the reversal capacity of these treatments are 22 and 28 fold, respectively, showing a higher reversal capacity compared to liposome encapsulated doxorubicin (9-fold).

Detailed Description Text (37):

Evidence of the role of the complexed cardiolipin-liposome-doxorubicin on multidrug resistance is demonstrated in FIG. 2. LZ cells were exposed to different concentrations of cardiolipin-liposome-complexed doxorubicin corresponding to a liposome/doxorubicin weight ratio of 11. As previously observed, drug is nearly all complexed to the liposome when incubated at 37.degree. C. and the liposomal doxorubicin preparation exerts a cytotoxic effect higher than that of free drug. When vincristine (an alkaloid anticancer agent) is mixed in the same conditions as Doxorubicin to cardiolipin-containing liposomes, no association between drug and liposome was formed and no increase in drug cytotoxicity was observed. The results demonstrate that cardiolipin-liposome complexed specifically doxorubicin and that this association is responsible for the increase of cytotoxicity against multidrug resistant cells.

Detailed Description Text (38):

In addition to the surprising advantages described above, the present invention also affords all of the advantages previously obtainable with liposomal anthracycline glycoside compositions. Notably, reference may be made to U.S. Pat. No. 4,419,348, which is incorporated herein in the entirety. Further, the following experiments illustrate the adaptability and versatility of the present method of making blank cardiolipin containing liposomes and their effective and efficient internalization of the drug inside the liposomes because of the strong affinity of the specific lipid to doxorubicin. The present method of preparing liposomes not only greatly simplifies the industrial manufacturing but actual patient treatment because of the usefulness of the method near the bed side of a patient as has been demonstrated by the spontaneous internalization of doxorubicin to the lyophilized and reconstituted liposomes.

CLAIMS:

1. A method for encapsulating in a cardiolipin-containing liposome an anthracycline glycoside, selected from the group consisting of doxorubicin and daunorubicin, consisting of the step of
 - a) mixing said cardiolipin-containing liposomes with an aqueous-based liquid comprising said anthracycline glycoside for a time sufficient to effect encapsulation of said anthracycline glycoside in said cardiolipin-containing

liposome,

whereby said anthracycline glycoside is encapsulated in said cardiolipin-containing liposome.

5. The method of claim 1, wherein at least about 75% of the anthracycline glycoside complexes with the cardiolipin-containing liposomes.

6. The method of claim 1, wherein at least about 75% of said anthracycline glycoside is encapsulated within said cardiolipin-containing liposome.

7. The method of claim 6, wherein at least about 95% of said anthracycline glycoside is encapsulated within said cardiolipin-containing liposome.

16. A method for encapsulating in a cardiolipin-containing liposome an anthracycline glycoside selected from the group consisting of doxorubicin and daunorubicin, consisting of the step of

a) rehydrating a mixture of (i) lyophilized cardiolipin-containing liposomes and (ii) said anthracycline glycoside in crystalline form by mixing therewith an aqueous-based liquid in amount sufficient to effect said rehydration,

whereby said anthracycline glycoside is encapsulated in said cardiolipin-containing liposome.

17. The method of claim 16, wherein at least about 75% of said anthracycline glycoside is encapsulated within said cardiolipin-containing liposome.

18. The method of claim 17, wherein at least about 95% of said anthracycline glycoside is encapsulated within said cardiolipin-containing liposome.

24. The method of claim 1, where said cardiolipin-containing liposome is lyophilized and said mixing effects rehydration of said lyophilized, cardiolipin-containing liposomes.

25. A method for encapsulating in a cardiolipin-containing liposome an anthracycline glycoside, selected from the group consisting of doxorubicin and daunorubicin, comprising the steps of

a) providing said cardiolipin-containing liposomes and said anthracycline glycoside; and

b) mixing said cardiolipin-containing liposomes with an aqueous-based liquid comprising said anthracycline glycoside for a time sufficient to effect encapsulation of said anthracycline glycoside in said cardiolipin-containing liposome,

whereby said anthracycline glycoside is encapsulated in said cardiolipin-containing liposome.

26. The method of claim 25, wherein at least about 75% of said anthracycline glycoside is encapsulated within said cardiolipin-containing liposomes.

27. The method of claim 25, wherein at least about 95% of said anthracycline glycoside is encapsulated within said cardiolipin-containing liposomes.

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